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Flavonoids from the Leaves of *Prunus spinosa* L.

by M. Olszewska* and M. Wolbiś

Department of Pharmacognosy, Medical University of Łódź, Muszyńskiego 1, 90-151 Łódź, Poland

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Two new flavonol glycosides, quercetin 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-arabinofuranoside and kaempferol 3-*O*-(2″-*O*-*E*-*p*-coumaroyl)-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside, were isolated from the leaves of *Prunus spinosa* L. The known compounds, kaempferol, quercetin, and their 3-arabinofuranosides, kaempferol 7-rhamnopyranoside, kaempferol 3,7-dirhamnopyranoside, and kaempferol 3-arabinofuranoside-7-rhamnopyranoside were also identified. Structural elucidation was performed by means of chemical methods and UV, IR, LSI MS, 1D and 2D NMR spectroscopy.

Key words: *Prunus spinosa*, *Rosaceae*, leaves, flavonoids, isolation

Prunus spinosa L. *(Rosaceae)* – blackthorn, is a popular medicinal plant growing in the north temperate zone [1], common in Poland [2]. Leaf extracts of *P. spinosa* have been used in traditional medicine for many purposes. In pharmacological studies (*in vivo*) the complex of flavonoids from blackthorn leaves decreased the capillary permeability and showed a significant anti-inflammatory, spasmolytic, diuretic and natriuretic effect and also very low toxicity [3]. In our previous work we have reported, that the leaves of *P. spinosa* are very rich source of flavonols and contain about 1.5% flavonoids, calculated as aglycones, and 2.5% calculated as glycosides by HPLC method [4]. That prompted us to detailed investigations of flavonoids in this plant material. From the blackthorn leaves several flavonoids: kaempferol, quercetin and their 3-*O*-α-L-arabinofuranosides, kaempferol 3- and 7-*O*-α-L-rhamnofuranosides [5,6], kaempferol 3,7-di-*O*-α-L-rhamnofuranoside and 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnofuranoside [7], and kaempferol 3-rhamno-4′-arabinoside [8] were isolated previously. The identification of the last compound was later questioned [3], the assumption being, it is in fact probably a mixture of kaempferol 3,7-dirhamnoside and kaempferol 3-arabinoside-7-rhamnoside. Moreover, to our knowledge, the occurrence of furanose form of rhamnose in the blackthorn glycosides is doubtful and there is a need of reexamination and/or structure revision for the latter compounds.

In the present work we describe the isolation and identification of nine flavonoids; two of them are new natural products.

*Corresponding author. Tel.: +(prefix)-42-6789727. Fax: +(prefix)-42-6788398.
E-mail address:molszewska@pharm.am.lodz.pl

RESULTS AND DISCUSSION

Pure flavonoids **1–9** were isolated from the leaves of *P. spinosa* by preparative column chromatography. Compounds **1–3** and **7, 8** were identified by spectral analysis and by comparisons with previously reported data for flavonols isolated from the blackthorn flowers [9]: kaempferol (**1**), quercetin (**2**), kaempferol 7-*O*-α-L-rhamnopyranoside (**3**), kaempferol and quercetin 3-*O*-α-L-arabinofuranosides (**7** and **8**, respectively).

Compounds **4** and **5**, after complete acid hydrolysis, gave L-rhamnose for **4**, the mixture of L-rhamnose and L-arabinose for **5** and kaempferol as aglycone. The glycosylation positions at C-3 and C-7 in both compounds, configuration and ring forms of sugar moieties were determined by UV, ^{1}H and ^{13}C NMR analyses [10–13]. Thus, the structure of **4** was confirmed as kaempferol 3,7-di-*O*-α-L-rhamnopyranoside and **5** as kaempferol 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside. In both cases the ring form of L-rhamnose is different from that suggested earlier for flavonoids isolated by Makarov *et al*. from the leaves of *P. spinosa* [7].

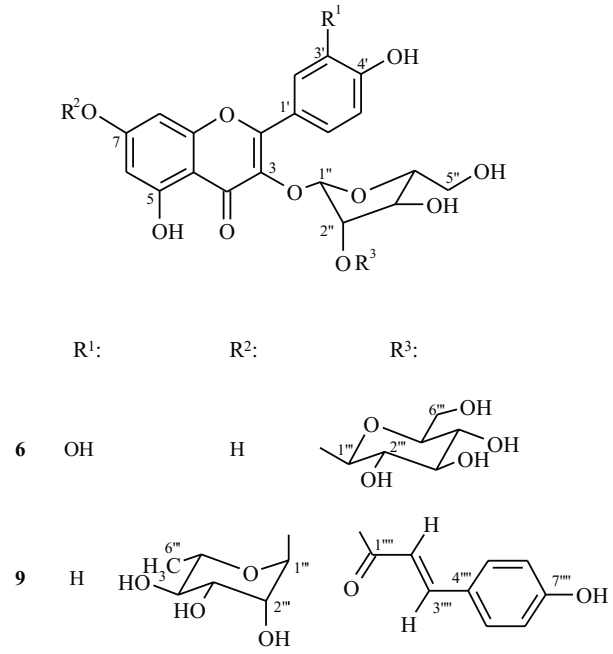


Figure 1

Flavonoids **6** and **9** are new natural products. Complete acid hydrolysis of **6** gave quercetin and two carbohydrates: L-arabinose and D-glucose. UV analysis [10] confirmed substitution of C-3-OH group of the aglycone only, and therefore indicated, that **6** is a monodesmoside. The negative ion LSI MS of **6** showed a quasi-molecular ion peak at m/z 595 $[M-H]^{-}$, corresponding to the molecular formula $C_{26}H_{28}O_{16}$ (con-

firmed by elemental analysis), and a fragmentation ion peak at m/z 433 [quer+ara-H]$^-$ indicated the loss of the glucosyl moiety, suggesting its terminal position. In the ^{1}H NMR spectrum of **6** five signals of aromatic protons, characteristic for quercetin and two signals of anomeric protons were found. The doublet of the glucose anomeric proton appeared at δ 4.45 ppm and showed the diaxial coupling constant $J_{1,2}$ = 7.8 Hz, thus confirming the terminal position and the β-configuration of D-glucosyl unit [12]. The presence of the anomeric proton signal of arabinose as a singlet at δ 5.87 ppm indicated the α-configuration of the L-arabinofuranosyl residue and suggested, by its significantly downfield shift (0.27 ppm in comparison with underivatized 3-*O*-α-L-arabinofuranosides) the glycosylation in position C-2 of L-arabinose [12]. The proposed structure of **6** was unequivocally confirmed by the 2D NMR spectroscopy (^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR experiments). The carbon resonance at δ 89.6 ppm correlated with the proton signal at δ 4.41 ppm, which was earlier identified, by ^{1}H-^{1}H COSY experiment, as the signal of H-2 of arabinose. Thus, the C-2″ signal of **6** was shifted downfield by 7.5 ppm compared with signal of the corresponding carbon atom in quercetin 3-*O*-α-L-arabinofuranoside [11]. However, the signal at δ 106.4 ppm, identified as C-1 of arabinose (HETCOR), was shifted upfield by 1.7 ppm. These data established the glycosylation position at C-2 of arabinosyl unit. Thus, **6** was regarded as quercetin 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-arabinofuranoside.

Compound **9**, after complete acid hydrolysis, gave kaempferol, L-arabinose, L-rhamnose, and an additional product, identified as *p*-coumaric acid. Mild alkaline hydrolysis of **9** led to *p*-coumaric acid and a deacylated compound identical to **5**. The UV spectrum of **9** confirmed the position of the linkages, between kaempferol and the other moieties at C-3 and C-7, and therefore indicated, that **9** is a bidesmoside [10]. The λ_{max} of band I appeared at 319 nm, due to the presence of an additional aromatic group in the side chain, probably a hydroxycinnamoyl unit [14]. In IR spectrum, apart from absorption bands characteristic for **5**, an additional signal of an ester group was observed at 1703 cm^{-1}. The negative ion LSI MS of **9** showed a quasimolecular ion peak at m/z 709 [M-H]$^-$, corresponding to the molecular formula $C_{35}H_{34}O_{16}$ (confirmed by elemental analysis). The presence of a fragmentation ion peak at m/z 563 [kaempf+rham+ara-H]$^-$, due to the loss of an acyl group, confirmed the structure of the hydroxycinnamoyl ester. In the ^{1}H NMR spectrum of **9** signals of six aromatic protons of kaempferol were observed, in addition to two doublets of *p*-disubstituted aromatic protons (J = 8.6 Hz) and two doublets of olefinic protons (J = 15.8 Hz), characteristic for *E*-*p*-coumaroyl unit [12]. The signals of anomeric protons of L-arabinose and L-rhamnose were found as singlets at δ 5.82 and 5.55 ppm, respectively, which indicated the α-configuration for both sugars and the substitution pattern of sugars and kaempferol, identical as in **5** [11], thus the occurrence of L-arabinosyl unit at C-3-OH, and L-rhamnosyl unit at C-7-OH group of the aglycone. The other signals in ^{1}H and ^{13}C NMR spectra were assigned by 2D NMR studies (^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR). The proton signal at δ 5.37 ppm (doublet, J = 3.1 Hz) identified as H-2 of arabinosyl residue (shifted by 1.2 ppm downfield in comparison to H-2″in **5**), correlated with the carbon signal at δ 83.8 ppm (HETCOR ex-

periment). However, protons H-1 and H-3 of arabinosyl unit correlated with carbons at δ 105.4 and 74.8 ppm, respectively. Thus, the signals of C-1″ and C-3″ in **9**, identified in this way, were shifted upfield by 2.7 and 2.3 ppm, respectively, and the signal of C-2″ by 1.7 ppm downfield, in comparison to the corresponding signals of **5**. These shifts are expected from the substitution effect of C-2 acylation of sugar moiety [12]. The chemical shifts of other protons and carbons of sugar moieties and the aglycone were similar for **9** and **5**. Thus, **9** was identified as kaempferol 3-*O*-(2″-*O*-*E*-*p*-coumaroyl)-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside.

In our investigations we have not found in *P. spinosa* leaves kaempferol 3-rhamno-4′-arabinoside, which was earlier reported [8] as the main flavonoid of this plant material and which was identified only by degradation studies. We found, the main flavonoids of blackthorn leaves are kaempferol 3,7-dirhamnoside and kaempferol 3-arabinoside-7-rhamnoside, and these compounds are very difficult to separation. So, we agree with Makarov *et al*. [3], that the compound isolated by Hőrhammer *et al*. [8] was in fact a mixture of these kaempferol glycosides.

Flavonoid arabinosides and other pentosides (xylosides, apiosides *etc*.) are relatively rare in nature [12,15]. Thus, the occurrence of several flavonol pentosides in *P. spinosa* leaves and also in the flowers [9,16] may be of great chemotaxonomic importance. Among flavonoid arabinosides rare are also acylated compounds [12], and to our knowledge, compound **9** is a second glycoside acylated with an aromatic moiety. Previously only 3-*O*-(2″-*O*-*E*-*p*-coumaroyl)-α-L-arabinofuranoside was isolated from *Picea koraiensis* needles [14] and from *P. spinosa* flowers [9].

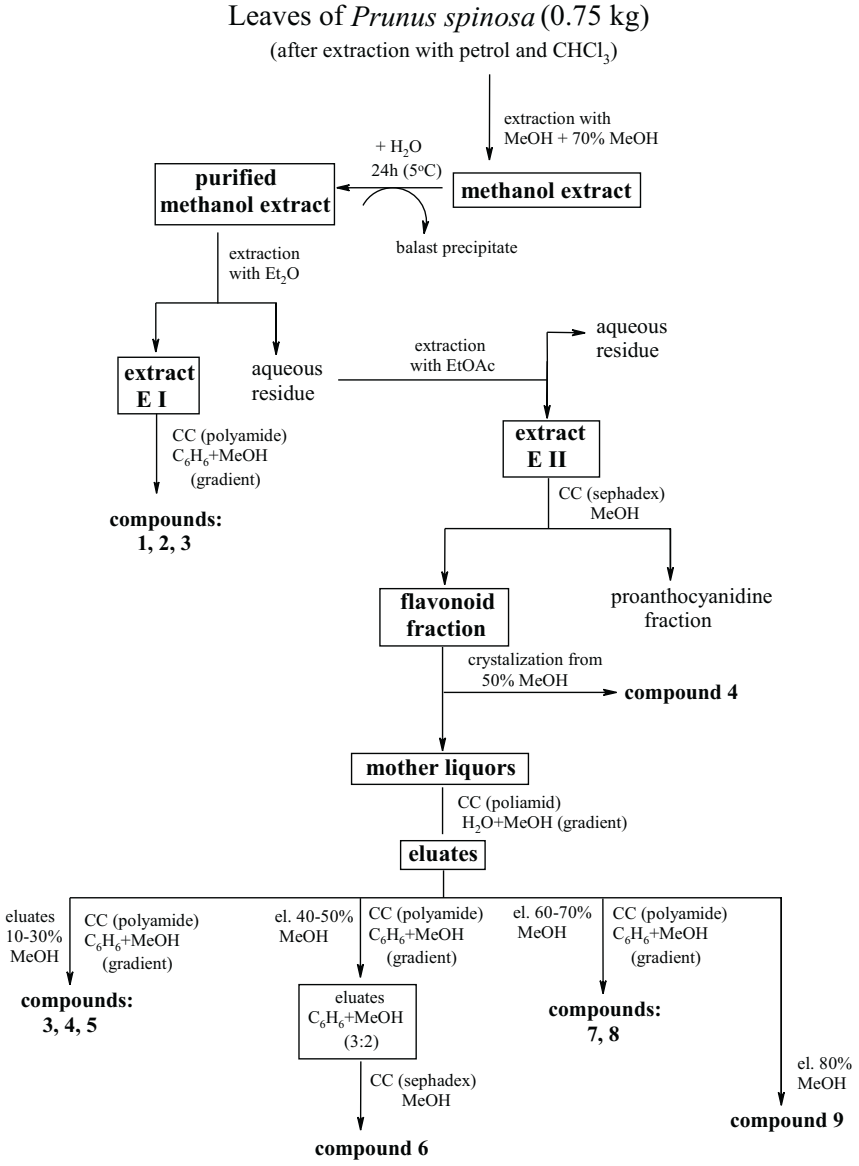
EXPERIMENTAL

General experimental procedures. Melting points (uncorr.) were determined on a Boetius apparatus. Optical rotations were measured on a Perkin Elmer 241 ME polarimeter. PC was carried out on Whatman No 1 paper with n-BuOH-HOAc-H_2O (4:1:5, upper phase) (system S-1), 15% HOAc (S-2), HCOONa-HCOOH-H_2O (10:1:200) (S-3); TLC on silica gel 60 precoated plates (Merck) with EtOAc-HCOOH-H_2O (18:1:1) (S-4), EtOH-25% NH_4OH-H_2O (20:1:4) (S-5); CC on polyamide SC-6 (MN) and Sephadex LH-20 (Fluka). Spectra were recorded using the following instruments: UV, Unicam SP 800 in MeOH and after addition of the usual shifts reagents, acc. to standard procedures [10]; IR, Mattson FTIR (in KBr); LSI MS, Finnigan MAT 95 (in gly., Cs^+, 13 keV); ^1H and ^{13}C NMR, Bruker DRX 500 MHz (in DMSO-d_6, TMS as int. standard). Elemental analyses were made with a Carlo Erba EA 1110 apparatus.

Plant material. Leaves of *P. spinosa* were collected in June 1996 from a natural habitat at Piwniczna (near Stary Sącz, southern Poland) and identified by Prof. M. Wolbiś. A voucher specimen (Fol.P.S.06.1996) is deposited at the Department of Pharmacognosy, Medical University of Łódź.

Extraction and isolation of the flavonoids. The dried and powdered leaves of *P. spinosa* (0.75 kg) were extracted with petrol and $CHCl_3$ (Soxhlet apparatus), followed by exhaustive extraction with MeOH and 70% aq. MeOH at the boiling temp. of the solvents. Combined methanol extracts were concentrated, suspended in hot distillet water, then cooled, and the balast precipitate (ppt.) was filtered off. The remaining filtrate (aq. solution) was subsequently extracted with Et_2O (E-I, 5.5 g) and EtOAc (E-II, 18.2 g). The obtained extracts were separated according to Scheme 1. E-I (2.0 g) was subjected to column chromatography (CC) on polyamide (eluent C_6H_6-MeOH with increasing amounts of MeOH) to yield **1** (45 mg), **2** (20 mg) and **3** (160 mg). E-II was chromatographed (CC) on sephadex (eluent MeOH) to afford two fractions: containing flavonoids (14.4 g) and proanthocyanidins (3.2 g). After crystalization from 50% aq.

MeOH, compound **4** (530 mg) was obtained from the flavonoid fraction. Mother liquors were chromatographed on polyamide (eluent H_2O-MeOH, with increasing gradient of MeOH). The fractions eluted with 10–30% and 60–70% aq. MeOH, were separately rechromatographed on polyamide (eluent C_6H_6-MeOH with increasing content of MeOH) to afford **3** (53 mg), **4** (64 mg), **5** (271 mg), and **7** (40 mg), **8** (70 mg), respectively. The 40–50% and 80% aq. MeOH fractions were rechromatographed on sephadex (eluent MeOH) to give **6** (10 mg) and **9** (60 mg), respectively.



Scheme 1. Isolation of the flavonoids from the leaves of *Prunus spinosa* L.

Hydrolyses experiments.

Complete acid hydrolysis of 3–9. Each glycoside (2 mg) was refluxed with 5% H2SO4 (6 ml) for 2 hr. Insoluble ppt. (aglycone) was filtered off, washed with water and identified by PC (S-1) and TLC (S-4) with authentic samples of kaempferol and quercetin. The filtrate was neutralized with $BaCO_3$. Precipitated $BaSO_4$ was filtered off. The sugars from the aq. solution were identified by PC and TLC (S-1, S-5) with authentic samples (detection with aniline phthalate at 105°C).

Alkaline hydrolysis of 9. Compound **9** (2 mg) was refluxed with 0.5% NaOH (5 ml) for 30 min at 60°C. The reaction mixture was acidified with 2% HCl to pH 3 and left for 3 h at 5°C. Insoluble ppt. (deacylated glycoside) was filtered off and identified by PC and TLC (S-1, 2, 4) with **5**. The filtrate was extracted with Et_2O. The Et_2O extract was washed with H_2O, evaporated to dryness and dissolved in MeOH. *p*-Coumaric acid was identified by PC (S-3) with an authentic sample (detection with 0.5% soln. of diazosulphanilic acid in 10% Na_2CO_3).

Kaempferol 3,7-di-*O*-α-L-rhamnopyranoside (4). Pale yellow needles, m.p. 193–197°C; PC R_fs: 0.66 (S-1), 0.73 (S-2); TLC Rf: 0.27 (S-4); UV (MeOH) λ_{max}: 265, 320sh, 346 nm; (MeOH+NaOMe) 247, 267, 300sh, 388 nm; (MeOH+$AlCl_3$) 237, 273, 301, 348, 398 nm; (MeOH+$AlCl_3$+HCl) 237, 273, 300, 345, 397 nm; (MeOH+NaOAc) 263, 322sh, 360, 390 nm; (MeOH+NaOAc+H_3BO_3) 263, 320sh, 347 nm; ^{1}H NMR δ, ppm: 12.59 (1H, s, OH-5), 7.79 (2H, d, J = 8.7 Hz, H-2′ and H-6′), 6.92 (2H, d, J = 8.7 Hz, H-3′ and H-5′), 6.77 (1H, d, J = 2.0 Hz, H-8), 6.44 (1H, d, J = 2.0 Hz, H-6), 5.55 (1H, d, J = 0.9 Hz, H-1‴), 5.30 (1H, d, J = 0.9 Hz, H-1″), 3.99 (1H, s, H-2″), 3.85 (1H, s, H-2‴), 3.64 (1H, dd, J_1 = 2.9 Hz, J_2 = 9.0 Hz, H-3″), 3.18–3.33 (2H, m, H-4″ and H-5″), 3.09–3.15 (3H, m, H-3‴, H-4‴ and H-5‴), 1.13 (3H, d, J = 6.0 Hz, 3H-6‴), 0.80 (3H, d, J = 5.3 Hz, 3H-6″); ^{13}C NMR data see Table 1.

Table 1. ^{13}C NMR data for compounds **4**, **5**, **6**, **9**, (125.7 MHz, δ, ppm).

C	4	5	9[a]	6[a]
2	156.1	156.0	156.0	156.8
3	134.6	133.7	133.3	133.5
4	178.0	177.9	177.7	177.9
5	161.0	160.8	160.8	161.2
6	99.5	99.4	99.4	99.2
7	161.7	161.7	161.7	164.7
8	94.6	94.6	94.6	94.1
9	157.9	157.3	157.2	157.8
10	105.8	105.6	105.6	104.3
1′	120.4	120.5	120.3	121.2
2′	130.8	131.0	130.9	116.0
3′	115.5	115.5	115.5	145.4
4′	160.2	160.1	160.1	148.8
5′	115.5	115.5	115.5	116.0
6′	130.8	131.0	130.9	122.2
	3-rha[b]:	3-ara[c]:	3-ara[c]:	3-ara[c]:
1″	101.9	108.1	105.4	106.4
2″	70.3	82.1	83.8	89.6

Table 1 (continuation)

3″	70.7	77.1	74.8	75.9
4″	71.2	86.4	86.0	85.8
5″	70.1	60.9	60.1	60.6
6″	17.5			
	7-rha[d]:	7-rha[d]:	7-rha[d]:	2″-glc[e]:
1‴	98.5	98.3	98.4	102.3
2‴	70.3	70.1	70.1	73.7
3‴	70.7	70.2	70.2	76.9
4‴	71.6	71.6	71.6	70.0
5‴	69.9	69.8	69.8	77.1
6‴	17.9	17.9	17.9	61.2
1⁗			165.6	
2⁗			113.3	
3⁗			145.9	
4⁗			124.9	
5⁗			130.6	
6⁗			115.8	
7⁗			160.3	
8⁗			115.8	
9⁗			130.6	

[a]Assignments were confirmed by ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR spectra, [b]3-rha and [d]7-rha = 3- and 7-*O*-α-L-rhamnopyranosyl, respectively, [c]3-ara = 3-*O*-α-L-arabinofuranosyl, [e]2″-glc = 3-*O*-β-D-glucofuranosyl.

Kaempferol 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside (5). Pale yellow needles, m.p. 196–198°C; PC R_fs: 0.71 (S-1), 0.70 (S-2); TLC Rf: 0.33 (S-4); $[\alpha]_D^{27}$: –271.4° (c 0.50, MeOH); UV (MeOH) λ_{max}: 245sh, 266, 320sh, 350 nm; (MeOH+NaOMe) 243, 270, 300sh, 345sh, 390 nm; (MeOH+$AlCl_3$) 255sh, 275, 302, 349, 400 nm; (MeOH+$AlCl_3$+HCl) 274, 300, 347, 398 nm; (MeOH+NaOAc) 264, 322sh, 356, 396 nm; (MeOH+NaOAc+H_3BO_3) 267, 320sh, 352 nm; IR ν_{max}: 1661.5 (C=O, γ-pyrone) cm^{-1}; ^{1}H NMR δ, ppm: 12.60 (1H, s, OH-5), 8.07 (2H, d, *J* = 8.9 Hz, H-2′ and H-6′), 6.89 (2H, d, *J* = 8.9 Hz, H-3′ and H-5′), 6.83 (1H, d, *J* = 1.9 Hz, H-8), 6.44 (1H, d, *J* = 1.9 Hz, H-6), 5.63 (1H, s, H-1″), 5.55 (1H, s, H-1‴), 4.15 (1H, m, H-2″), 3.83 (1H, brs, H-2‴), 3.72–3.73 (1H, m, H-3″), 3.61–3.64 (1H, m, H-3‴), 3.53–3.56 (1H, m, H-4″), 3.24–3.44 (4H, m, 2H-5″, H-4‴ and H-5‴), 1.11 (3H, d, *J* = 6.1 Hz, 3H-6‴); ^{13}C NMR data see Table 1; LSI MS (negative mode) m/z (rel. nt.): 563 $[M-H]^-$ (100), 285 $[M-H-278]^-$ (6).

Quercetin 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-arabinofuranoside (6). Amorphous yellow powder, m.p. 196–200°C; PC R_fs: 0.48 (S-1), 0.52 (S-2); TLC R_f: 0.07 (S-4); UV (MeOH) λ_{max}: 256, 268sh, 300sh, 355 nm; (MeOH+NaOMe) 271, 327, 405 nm; (MeOH+$AlCl_3$) 273, 305sh, 432 nm; (MeOH+$AlCl_3$+HCl) 269, 302sh, 362, 396 nm; (MeOH+NaOAc) 270, 323sh, 394 nm; (MeOH+NaOAc+H_3BO_3) 268, 324sh, 386 nm; ^{1}H NMR δ, ppm: 12.55 (1H, s, OH-5), 7.52 (1H, dd, J_1 = 2.2 Hz, J_2 = 8.4 Hz, H-6′), 7.44 (1H, d, *J* = 2.2 Hz, H-2′), 6.84 (1H, d, *J* = 8.4 Hz, H-5′), 6.40 (1H, d, *J* =

2.0 Hz, H-8), 6.19 (1H, d, J = 2.0 Hz, H-6), 5.87 (1H, s, H-1″), 4.45 (1H, d, J = 7.8 Hz, H-1‴), 4.41 (1H, d, J = 2.9 Hz, H-2″), 3.90 (1H, dd, J_1 = 3.0 Hz, J_2 = 6.6 Hz, H-3″), 3.63 (1H, d, J = 11.5 Hz, H-$6^{'''}_b$), 3.51 (1H, brd, J = 9.8 Hz, H-$6^{'''}_a$), 3.44 (1H, m, H-4″), 3.23–3.37 (2H, m, 2H-5″), 3.13–3.18 (3H, m, H-3‴, H-4‴ and H-5‴); ^{13}C NMR data see Table 1; LSI MS (negative mode) m/z (rel. nt.): 595 $[M-H]^-$ (100), 433 $[M-H-162]^-$ (1), 301 $[M-H-294]^-$ (3); Anal. Calcd. for $C_{26}H_{28}O_{16}$: C 52.35, H 4.70. Found: C 52.23, H 4.65.

Kaempferol 3-*O*-(2″-*E*-*p*-coumaroyl)-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside (9). Pale yellow needles, m.p. 208–210°C; PC R_fs: 0.90 (S-1), 0.42 (S-2); TLC R_f: 0.49 (S-4); $[\alpha]_D^{27}$: –121.7° (c 0.49, MeOH); UV (MeOH) λ_{max}: 269, 298sh, 319, 350 nm; (MeOH+NaOMe) 250sh, 273, 300sh, 377 nm; (MeOH+$AlCl_3$) 277, 303, 325, 400 nm; (MeOH+$AlCl_3$+HCl) 278, 304, 325, 396 nm; (MeOH+NaOAc) 269, 300sh, 323, 374 nm; (MeOH+NaOAc+ H_3BO_3) 268, 300sh, 322, 352 nm; IR ν_{max}: 3308 (OH), 2943 (CH), 1703 (C=O, ester), 1658 (C=O, γ-pyrone), 1627, 1602, 1514, 1496 and 1440 (aromatic rings), 1373, 1352, 1207, 1184, 1167, 1110, 1063, 1027, 964, 919, 871, 834, 808, 731, 517 cm^{-1}; ^{1}H NMR δ, ppm: 12.50 (1H, s, OH-5), 8.06 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.62 (1H, d, J = 15.9 Hz, H-3‴), 7.58 (2H, d, J = 8.6 Hz, H-5‴ and H-9‴), 6.90 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 6.84 (1H, d, J = 1.9 Hz, H-8), 6.79 (2H, d, J = 8.6 Hz, H-6‴ and H-8‴), 6.44 (1H, d, J = 15.8 Hz, H-2‴), 6.43 (1H, d, J = 1.9 Hz, H-6), 5.82 (1H, s, H-1″), 5.55 (1H, s, H-1‴), 5.37 (1H, d, J = 3.1 Hz, H-2″), 4.01 (1H, m, H-3″), 3.83 (1H, brs, H-2‴), 3.67 (1H, m, H-4″), 3.63 (1H, dd, J_1 = 2.9 Hz, J_2 = 9.2 Hz, H-3‴), 3.42 (2H, dd, J_1 = 6.2 Hz, J_2 = 9.2 Hz, H-4‴ and H-5‴), 3.27–3.34 (2H, m, 2H-5″), 1.11 (3H, d, J = 6.1 Hz, 3H-6‴); ^{13}C NMR data see Table 1; LSI MS (negative mode) m/z (rel. nt.): 709 $[M-H]^-$ (100), 563 $[M-H-146]^-$ (44), 431 $[M-H-]^-$ (3), 285 $[M-H-424]^-$ (3); Anal. Calcd. for $C_{35}H_{34}O_{16}$: C 59.15, H 4.79. Found: C 59.12, H 4.76.

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